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Anti-oxidant capacity and anti-tumor T cell function: A direct correlation

Pravin Kesarwani[1], Krishnamurthy Thyagarajan[1], Shilpak Chatterjee[1], Viswanathan Palanisamy[2], and Shikhar Mehrotra[1,*]

[1]Department of Surgery; Medical University of South Carolina; Charleston, SC USA; [2]Oral Health Sciences; Medical University of South Carolina; Charleston, SC USA

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Improving persistence and sustained function of effector $CD8^+$ T cell response is key for achieving significant tumor control in adoptive T cell immunotherapy protocols. Our recent report shows that high anti-oxidant property is central to potent anti-tumor effector T cells, and directly correlates to $CD62L^{hi}$ central memory, low glycolytic and low mitochondrial membrane potential phenotype, all of which may be linked and contribute to better tumor control.

Adoptive cell transfer (ACT) therapy is one of the promising approaches for treatment of melanoma. However, *ex vivo* quantitative expansion of the anti-tumor T cells does result in effector population with heterogeneous phenotype, a majority of which is qualitatively exhausted and exhibits poor persistence or susceptibility to oxidative stress in a tumor microenvironment. While $CD8^+$ T cells with central memory phenotype (T_{CM}) have been shown to persist better and exhibit better tumor control, the factors that result in their increased persistence in oxidative tumor microenvironment have not been comprehensively addressed.

Given the earlier reports that showed the reduced T helper (Th)-1 cytokine secretion by antigen activated/memory $CD45RO^+$ T cells under oxidative stress,[1] and enhanced tolerance of regulatory T cells to oxidative stress due to increased thioredoxin-1 levels,[1] we reasoned that anti-oxidant levels may be differentially expressed between T_{CM} *vs.* T_{EM} cells, and could be responsible for differences in anti-tumor potential. Our data shows that *ex vivo* activated tumor epitope reactive T cells with $CD62L^{hi}$ T_{CM}-like phenotype also exhibit higher expression of free sulfhydryl groups (–SH; reduced groups also called thiol) at cell surface (c-SH), intracellular glutathione (iGSH), and anti-oxidant enzymes as compared to the $CD8^+$ T cells with effector memory (T_{EM}) phenotype. Importantly, circulating human T cells from normal healthy individuals that were FACS sorted for $CD62L^{hi}$ *vs.* $CD62L^{lo}$ expression also displayed similar differences in anti-oxidant capacity. Increased anti-oxidant capacity of T_{CM} cells inversely correlated with the generation of reactive oxygen and nitrogen species, proliferative capacity and glycolytic enzyme levels. Notably, T cells pretreated with thiol donors, such as N-acetyl cysteine (L-NAC) or mTOR inhibitor rapamycin, upregulated thiol levels and antioxidant genes. Further, transferring FACS sorted tumor epitope reactive activated $CD8^+$ T cell populations on the basis of c-SH expression showed better *in vivo* persistence and concomitant anti-tumor immunity. Our results suggest that higher level of c-SH and anti-oxidant enzyme levels are of key importance for achieving T cell mediated tumor control.

While our study used the T cells that are inherently high on c-SH expression after *ex vivo* manipulation, deciphering the culture conditions that promote the expansion of c-SH high cells could be key for improving T cell immunotherapy.[2] Our study highlights that using Rapamycin – a drug that is known to enhance CD62L expression,[3] also increases anti-oxidant capacity of the T cells. It is likely that in addition to its role as mTOR inhibitor and regulating metabolic commitment of a T cell, rapamycin pre-treated T cells persist better in a tumor microenvironment and exhibit improved anti-tumor function because increased capacity to resist oxidative stress.[3] Although, we noticed that while $CD62L^{hi}$ cells and cells treated with thiol donor L-NAC showed the similar metabolic profile, (i.e. lower oxidative-phosphorylation (OXPHOS) and glycolysis), they also exhibited lower activation of mTOR pathway (as observed by reduced phosphorylation of S6). Thus, an inverse correlation between the mTOR status and glycolytic/OXPHOS commitment with anti-oxidant state in T cells could be envisioned. While this observation is line with the recent study that showed pre-treating tumor reactive T cells with glycolytic inhibitor improved tumor response,[4] it also identifies that interchangeable OXPHOS and glycolytic commitment in T cells shown earlier,[5] could in fact be due to dynamic T cell phenotype associated with differential metabolic activity in anti-$oxidant^{hi}CD62L^{hi}$-T_{CM} like cells as compared to anti-$oxidant^{lo}CD62L^{lo}$-T_{EM} like cells. In addition, if the differences observed in metabolic commitment between activated T cell subsets were due to cytokines milieu or antigen presenting cells that have been shown to modulate redox molecules[6-8] needs further investigation.

Recent advances in redox proteomics combined with mass spectrometry and affinity chemistry-based methodologies have contributed in a significant way to provide a better understanding of protein oxidative modifications, and will also be needed to elucidate the differences in

*Correspondence to: Shikhar Mehrotra; Email: mehrotr@musc.edu
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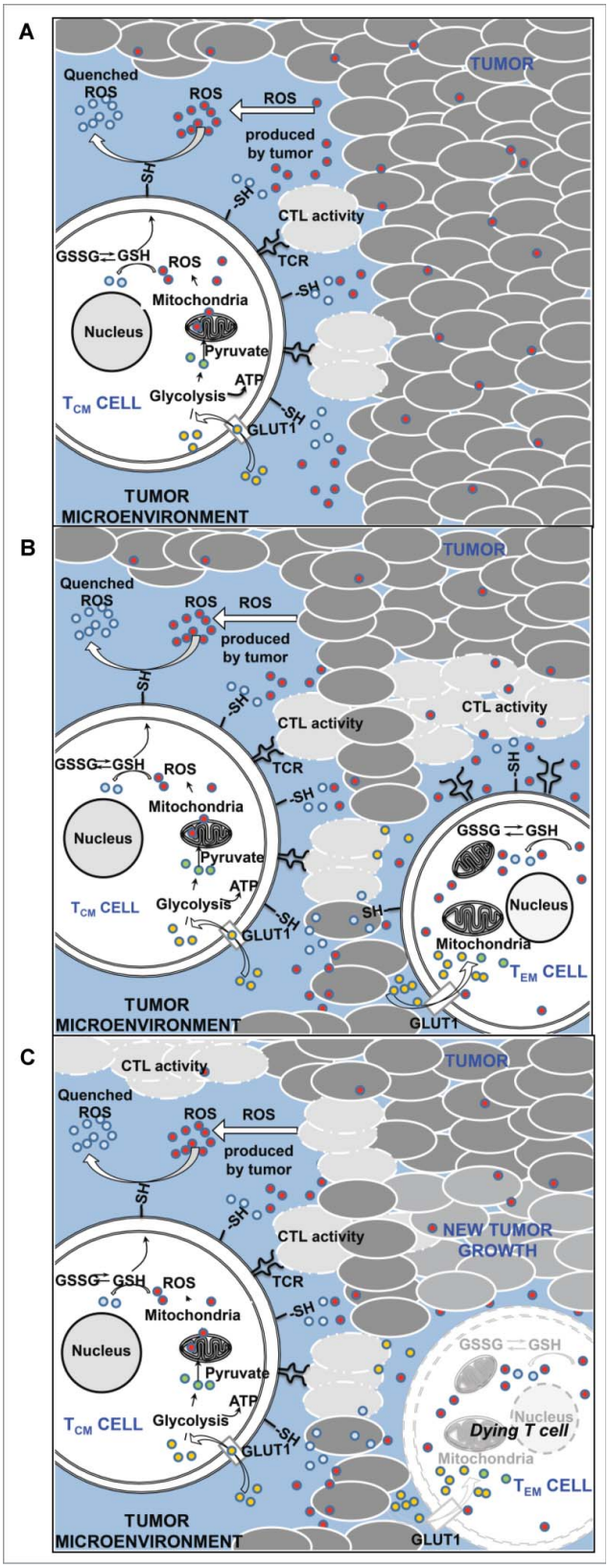

reduced vs. oxidative proteins in T cells subsets that would contribute to differences in persistence and function. A recent study performed comparative proteome analysis between naïve $CD45RA^{+}$ and effector/memory $CD45RO^{+}$ T cells showed increased expression of proteins involved in cytoskeletal organization (actins, tubulins, talin-1, vinculin), energy metabolism (enolase, phosphogycerate kinase1), and anti-oxidants (superoxide dismutase, thioredoxin1).[9] In addition, T cell subsets exposed to low dose hydrogen peroxide to mimic oxidative tumor microenvironment showed that the expression of various pathway proteins was differentially affected due to differences in susceptibility to oxidative stress. Thus, it is likely that due to lower reduced –SH group and anti-oxidant capacity, the adoptively transferred $c\text{-}SH^{lo}$ T cells in our study succumbed to reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) that are produced and released in high amounts by tumor cells as well as by tumor-infiltrating bystander cells like activated granulocytes, tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC). Further, a close association of better tumor control with increased autoimmunity could not be negated when using $c\text{-}SH^{hi}$ T cells reactive to "self", but, tumor epitope, as differences in thiol has

Figure 1. Schematic diagram depicting correlation between thiol (–SH) distribution on T_{CM} and T_{EM} cell, persistence and tumor control. Tumor cell produce high amount of ROS (red dots), which can be quenched (blue dots) by T_{CM} cells resulting in improved persistence. GSH (reduced glutathione) and GSSG (oxidized glutathione dimer) inside a T cells could replenish c–SH groups through glutathionylation of antioxidant enzymes such as thioredoxin, glutaredoxin, etc. (**A**) T_{CM} cells have higher expression of thiols on cell surface (c–SH) and GSH, however, they have lower CTL activity. (**B**) On the other hand T_{EM} cells show much lower c–SH and GSH but much higher CTL activity. T_{CM} cells also show lower glucose uptake (yellow dots) using glucose transporter (GLUT1). (**C**) In a tumor microenvironment T_{EM} cells are not able to survive longer due to decreased ability to quench ROS and succumb in an oxidative tumor microenvironment leading to tumor growth. However, T_{CM} cells can survive tumor mediated oxidative stress and can effectively control tumor growth long-term.

also been associated with susceptibility to autoimmune arthritis.[10]

In conclusion, we propose that anti-oxidant capacity (as determined by c-SH, iGSH or anti-oxidant enzyme levels) should be considered as biomarkers for identifying effector T cells that would be potentially long-lived and attain significantly long-term tumor regression (as depicted in **Fig. 1**). In addition, culture strategies to expand tumor epitope reactive effector T cells by including cytokines or immunomodulatory agents that intrinsically affect the anti-oxidant level in T cells could also be potentially useful in tumor immunotherapy.

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